

wound healing, as discussed in COCKERILL et al., "Angiogenesis: Model and Modulators", Int Rev

Cytol, 159: 113-160 (1995); and FOLKMAN et al., "Angiogenesis", J Biol Chem, 267:10931-10934

*cont.* (1992), the disclosures of which are herein incorporated by reference in their entireties.---

**Please replace the first full paragraph on page 2 with the following new paragraph:**

---Abnormal or uncontrolled angiogenesis is a prominent feature in various disease states including diabetic retinopathy, arthritis, hemangiomas, psoriasis, etc. In addition, abnormal capillary growth is an important step during the transition from hyperplasia to neoplasia and metastasis, and is critical for the growth and maintenance of many types of benign and malignant tumors, as disclosed in FOLKMAN, "The Role of Angiogenesis in Tumor Growth", Seminar in Cancer Biol., 3:65-71 (1992); FOLKMAN et al., "Induction of Angiogenesis During the Transition from Hyperplasia to Neoplasia", Nature 339: 58-61 (1989); FRIEDLANDER et al., "Definition of Two Angiogenic Pathways by Distinct  $\alpha_v$  Integrins", Science, 270:1500-1502 (1995); LIOTTA et al., "Cancer Metastasis and Angiogenesis: an Imbalance of Positive and Negative Regulation", Cell, 64:327-336 (1991); SACLARIDES et al., "Tumor Angiogenesis and Rectal Carcinoma", Dis. Colon Rectum, 37:921-926 (1994); and SHWEIKI et al., "Patterns of Expression of Vascular Endothelial Factor (VEGF) and VEGF Receptors in Mice Suggest a Role in Hormonally Regulated Angiogenesis", J. Clin. Invest., 91:2235-2243 (1993), the disclosures of which are herein incorporated by reference in their entireties.---

**Please replace the paragraph bridging pages 2-3 with the following new paragraph:**

It has been observed that in human breast carcinomas the intratumoral endothelial cell proliferation index (mean 2.7%) is 45-fold greater than that of the surrounding benign breast, as disclosed in VARTANIAN et al., "Correlation of Intratumoral Endothelial Cell Proliferation with Microvessel Density (Tumor Angiogenesis) and Tumor Cell Proliferation in Breast Carcinoma", Am. J. Pathol., 144:1188-1194 (1994), the disclosure of which is herein incorporated by reference in its entirety. The value of 2.7% however is unexpectedly low, especially given the much higher intratumoral microvascular density compared to adjacent benign stroma. This has suggested that a significant component of the angiogenic process is due to endothelial cell migration, capillary budding, establishment of capillary loops, and/or neovascular remodeling, as disclosed FOLKMAN et al., "Angiogenic Factors", Science, 235:442-447 (1987); FURCHT, "Critical Factors Controlling Angiogenesis: Cell Products, Cell Matrix, and Growth Factors", Lab. Invest., 55:505-509 (1986); DENEKAMP, "Angiogenesis, Neovascular Proliferation and Vascular Pathophysiology as Targets for Cancer Therapy", Br. J. Radiol., 66:181-196 (1993); and MAHADEVAN et al., "Metastasis and Angiogenesis", Rev. Oncol., 3:97-103 (1990), the disclosures of which are herein incorporated by reference in their entireties.

**Please replace the paragraph bridging pages 4-5 with the following new paragraph:**

---The direct evidence, supporting that tumor growth is angiogenesis dependent, is that various methods of inhibiting angiogenesis, which are not cytostatic to tumor cells *in vitro*, inhibit tumor growth *in vivo*, as disclosed in FOLKMAN, "Angiogenesis and its Inhibition", Important

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Advances in Oncology, pp. 42-62 (1985); FOLKMAN, "Clinical Applications of Research on Angiogenesis", N. Engl. J. Med., 333:1757-1763 (1995); HARRIS et al., "Gene Therapy Through Signal Transduction Pathways and Angiogenic Growth Factors as Therapeutic Targets in Breast Cancer", Cancer, 74:1021-1025 (1994); INGBER et al., "Synthetic Analogues of Fumagillin that Inhibit Angiogenesis and Suppress Tumor Growth", Nature, 348:555-557 (1990); GROSS et al., "Modulation of Solid Tumor Grown *in vivo* by bFGF", Proc. Am. Assoc. Cancer Res., 31:79 (#469) (1990); HORI et al., "Suppression of Solid Tumor Growth by Immunoneutralizing Monoclonal Antibody Against Human Basic Fibroblast Growth Factor", Cancer Res., 51:6180-6184 (1991); KIM et al., "Inhibition of Vascular Endothelial Growth Factor-induced Angiogenesis Suppresses Tumor Growth *in vivo*", Nature, 362:841-844 (1993); MILLAUER et al., "Glioblastoma Growth Inhibited *in vivo* by a Dominant-negative Flk-1 Mutant", Nature, 367:576-579 (1994); BROOKS, "Integrin  $\alpha\beta 3$  Antagonists Promote Tumor Regression by Inducing Apoptosis of Angiogenic Blood Vessels", Cell, 79:1157-1164 (1994); and NICOSIA et al., "Interactions Between Newly Formed Endothelial Channels and Carcinoma Cells in Plasma Clot Culture", Clin. Exp. Metastasis, 4:91-104 (1986), the disclosures of which are herein incorporated by reference in their entireties.---

**Please replace the paragraph bridging pages 6-7 with the following new paragraph:**

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---The process of tumor neovascularization shares many features with normal wound healing, as disclosed in DVORAK, "Tumors: Wounds that do not Heal. Similarities between Tumor Stroma Generation and Wound Healing", N. Engl. J. Med., 315:1650-1659 (1986), the disclosure of which is herein incorporated by reference in its entirety, and is likely mediated by similar and specific

angiogenic molecules (e.g., VEGF), which are released by the tumor cells and/or host immune cells into the stroma or possibly mobilized from a bound inactive state within the tumor stroma (e.g., FGF-1), as disclosed in FOLKMAN et al. (1987); FOLKMAN (1995); and MOSCATELLI et al. (1981), all cited above, the disclosures of which are herein incorporated by reference in their entireties. In addition to tumor cells, inflammatory cells may also be important in tumor angiogenesis. Stimulated macrophage can secrete angiogenic factors, such as TGF $\alpha$ , angiotropin, TNF $\alpha$ , and bFGF/FGF-2, as disclosed in FOLKMAN (1995), cited above; POLVERINI et al., "Induction of Neovascularization *in vivo* and Endothelial Proliferation *in vitro* by Tumor Associated Macrophages", Lab. Invest., 51:635-642 (1984); BAIRD et al., "Immunoreactive Fibroblast Growth Factor in Cells of Peritoneal Exudate Suggests its Identity with Macrophage-derived Growth Factor", Biochem. Biophys. Res. Commun., 126:358-364 (1985); FRATER-SCHRODER et al., "Tumor Necrosis Factor Type a, a Potent Inhibitor of Endothelial Cell Growth *in vitro*, is Angiogenic *in vivo*", Proc. Natl. Acad. Sci (USA), 84:5277-5281 (1987); LEIBOCH et al., "Macrophage-induced Angiogenesis Mediator than Epidermal Growth Factor", Science, 232:1250-1253 (1987); SCHREIBER et al., "Transforming Growth Factor-alpha: a More Potent Angiogenic Mediator than Epidermal Growth Factor", Science, 232:1250-1253 (1986); HOCKEL et al., "Purified Monocyte-derived Angiogenic Substance (Angiotropin) Induces Controlled Angiogenesis Associated with Regulated Tissue Proliferation in Rabbit Skin", J. Clin. Invest., 82:1075-1090 (1988); and FOLKMAN, "A Heparin-binding Angiogenic Protein - Basic Fibroblast Growth Factor - is Stored within Basement Membrane", Am. J. Pathol., 130:393-400 (1988), the disclosures of which are herein incorporated by reference in their entireties.---

**Please replace the first full paragraph of page 7 with the following new paragraph:**

---Clearly, many tumors have associated macrophages, which may amplify tumor angiogenesis, especially when activated by high intratumoral lactate levels caused by tumor hypoxia, as disclosed in FOLKMAN (1988), cited above, the disclosure of which is herein incorporated by reference in its entirety. Also, some human tumors are infiltrated by mast cells, as disclosed in SMOLIN, "Lymphatic Drainage from Vascularized Rabbit Cornea", Am J. Ophthalmol., 72:147-151 (1971); and KESSLER et al., "Mast Cells and Tumor Angiogenesis", Intern. J. Can., 18:703-709 (1976), the disclosures of which are herein incorporated by reference in their entireties. Mast cells are rich in heparin, a substance known to mobilize bFGF/FGF-2 from the extracellular matrix, protect it from degradation, and potentiates its angiogenic effects, as disclosed in THORNTON et al., "Human Endothelial Cells: Use of Heparin in Cloning and Long-term Serial Cultivation, Science, 222:623-625 (1983), the disclosure of which is herein incorporated by reference in its entirety. Furthermore, when tumors are implanted in mast cell deficient mice (W/W<sup>y</sup>), angiogenesis and tumor growth are inhibited to less than 60% of that observed in mice having normal mast cells numbers, as disclosed in DETHLEFSEN et al., "Tumor Growth and Angiogenesis in Wild Type and Mast Cell Deficient Mice", FASEB J., 4:A623 (1990), the disclosure of which is herein incorporated by reference in its entirety. Tumor angiogenesis and tumor growth however have increased when these mast cell deficient mice are injected with exogenous mast cells along with the original bolus of tumor cells. Finally, stimulated tumor-infiltrating lymphocytes may also play a role in tumor angiogenesis by secreting cytokines that activate other inflammatory cell types, and/or chemo-attractants for other immune cells.---

**Please replace the paragraph bridging pages 7-8 with the following new paragraph:**

---Angiogenesis is a complex biochemical process, and it is often difficult to study the molecular mechanism *in vivo* due to interference by a multitude of factors. The exact molecular details of angiogenesis (normal or abnormal) are not fully understood. It is known, however, that the sequence of events of angiogenesis involve DNA synthesis and vascular remodeling. Angiogenesis occurs in stages that orchestrate a network of cooperative interactions which include: (I) the initiation phase, characterized by increased cell membrane permeability; (ii) progression, constituted by the production of proteolytic enzymes that degrade the extracellular matrix and promote endothelial cell migration, and the entry of cells into either a proliferative or an apoptotic response; (iii) differentiation into new vessels; and (iv) the stabilization and maturation of vessels by mediator molecules that recruit mesenchymal cells to vessel walls, as discussed in COCKERILL et al. (1995); KLAGSBRUN et al. (1990); and SHWEIKI et al. (1993), all cited above, the disclosures of which are herein incorporated by reference in their entireties.---

**Please replace the paragraph bridging pages 8-9 with the following new paragraph:**

---Although the list of factor(s) and/or cell(s) causing tumor angiogenesis remains incomplete, the current leading candidates for this role include bFGF/FGF-2 and VEGF, as disclosed in KANDEL et al., "Neovascularization is Associated with a Switch to the Export of bFGF in the Multi-step Development of Fibrosarcoma", Cell, 66:1095-1104 (1991); NGUYEN et al., "Elevated Levels of the Angiogenic Peptide Basic Fibroblast Growth Factor in Urine of Bladder Cancer Patients", J. Natl. Cancer Inst., 85:241-242 (1993); and HORI et al., "Suppression of Solid Tumor

Growth by Immunoneutralizing Monoclonal Antibody Against Human Basic Fibroblast Growth Factor", Cancer Res., 51:6180-6184 (1991), the disclosures of which are herein incorporated by reference in their entireties. Other possible angiogenic factors include: FGF-1, TGF $\alpha$ , TGF $\beta$ , platelet-derived endothelial cell growth factor (PD-ECGF), vascular permeability factor (VPF), folliculostellate-derived growth factor (FSDGF), granulocyte colony stimulating factor, placental growth factor, interleukin-8, hepatocyte growth factor, angiotropin, angiogenin, and TNF $\alpha$ , as disclosed in FOLKMAN et al. (1995); FOLKMAN (1995); and MOSCATELLI (1981), all cited above, the disclosures of which are herein incorporated by reference in their entireties. The amino acid sequences of VEGF, VPF, and FSDGF are nearly identical and likely represent the same substrate. In fact, VEGF is often designated VPF/VEGF. It has been shown in a variety of solid tumor types that tumor cells express high levels of VEGF protein and mRNA. In contrast, tumor endothelial cells express VEGF protein but not VEGF mRNA. Yet, the same endothelial cells express high levels of mRNA for the VEGF receptors Flt-1 and KDR, indicating that the endothelial-cell staining likely reflects binding of VEGF protein secreted by adjacent tumor cells. Moreover, VEGF has shown to induce in endothelial cells expression of plasminogen activator, plasminogen activator inhibitor, interstitial collagenase, and procoagulant activity, as disclosed in BROWN et al., "Increased Expression of Vascular Permeability Factor (Vascular Endothelial Growth Factor) and its Receptors in Kidney and Bladder Carcinomas", Am J. Pathol., 143:1255-1262 (1993), the disclosure of which is herein incorporated by reference in its entirety. VEGF promotes extravasation of plasma fibrinogen, leading to fibrin deposition within the tumor matrix, a process that promotes the ingrowth of macrophages, fibroblasts, and endothelial cells, as disclosed in SENGER et al.,

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"Vascular Permeability Factor (VPF, VEGF) in Tumor Biology", Can Met Rev., 12:303-324 (1993), the disclosure of which is herein incorporated by reference in its entirety. In addition, it has been suggested that VEGF and bFGF/FGF-2 act in a synergistic manner to cause tumor angiogenesis, as disclosed in GOTO et al., "Synergistic Effects of Vascular Endothelial Growth Factor and Basic Fibroblast Growth Factor on the Proliferation and Cord Formation of Bovine Capillary Endothelial Cells within Collagen Gels", Lab. Invest., 69:508-517 (1993), the disclosure of which is herein incorporated by reference in its entirety. ---

Please replace the paragraph bridging pages 9-11 with the following new paragraphs:

---Various low molecular weight, non-peptide angiogenic factors have also been reported. These include 1-butyryl-glycerol, prostaglandins E1 and E2 (PEG1 and PEG2), nicotinamide, adenosine, nitric oxide, hyaluronic acid degradation products, an arachidonic acid metabolites named 12(R)-hydroxyeicosatrienoic acid (12[R]-HETrE), 8Br-cAMP, estrogens (17 $\beta$ -estradiol), as disclosed in FOLKMAN et al. (1987), cited above; FOLKMAN (1995), cited above; LEIBOVICH et al., "Production of Angiogenic Activity by Human Monocytes Requires an L-arginine/nitric oxide-synthase-dependent Effector Mechanism", Proc. Natl. Acad. Sci (USA), 91:4190-4194 (1994); LANIADO-SCHWARTZMAN et al., "Activation of Nuclear Factor Kappa B and Oncogene Expression by 12(R)-hydroxyeicosatrienoic acid, an Angiogenic Factor in Microvessel Endothelial Cells", J. Biol. Chem., 269:2432-2437 (1994); BANERJEE, "Microenvironment of Endothelial Cell Growth and Regulation of Protein N-glycosylation", Indian J. Biochem. Biophys., 25:8-13 (1988); and BANERJEE et al., "Biphasic Estrogen Response on Bovine Adrenal Medulla Capillary

Endothelial Cell Adhesion, Proliferation and Tube Formation", Mol. Cell Biochem., 177:97-105 (1997). When endothelial cells are stimulated by 12(r)-HETrE, the proto-oncogenes *c-myc*, *c-jun*, and *c-fos* are activated, as disclosed in LANIADO-SCHWARTZMAN et al. (1988), cited above, the disclosure of which is incorporated herein by reference in its entirety.

Inactivation of a suppressor gene resulting in loss of an angiogenic suppressor substance may allow tumor angiogenesis to proceed. Indeed, the switch to active angiogenesis and the rate of the angiogenic process are likely the net effect of both stimulatory and inhibitory factors. For example, it has been shown that inactivation of a suppressor gene during carcinogenesis results in increased angiogenesis that parallels increased tumorigenicity, as disclosed in BOND et al., "Replacement of Residues of 8-22 of Angiogenin with 7-21 of RNASE-A Selectively Affects Protein-synthesis Inhibition and Angiogenesis", Biochemistry, 29:3341-3349 (1990); and BOUCK et al., "Coordinate Control of Anchorage Independence, Actin Cytoskeleton and Angiogenesis by Human Chromosome 1 in Hamster-human Hybrids", Cancer Res., 46:5101-5105 (1986), the disclosures of which are herein incorporated by reference in their entireties. During this process there is a 10-fold decrease in the secretion of an angiogenesis inhibitor 140 kDa glycoprotein, thrombospondin, as disclosed in RASTINEJAD et al., "Regulation of the Activity of a New Inhibitor of Angiogenesis by a Cancer Suppressor Gene", Cell, 56:345-355 (1989), the disclosure of which is herein incorporated by reference in its entirety.

Somatic hybrid cells produced by fusion of MCF-7 human breast carcinoma cells with normal immortalized human mammary epithelial cells are suppressed in their ability to form tumors in nude mice, as disclosed in ZAJCHOWSKI et al., "Suppression of Tumor-forming Ability and

Related Traits in MCF - 7 Human Breast Cancer Cells by Fusion with Immortal Mammary Epithelial Cells", Proc. Natl. Acad. Sci. (USA), 87:2314-2318 (1990), the disclosure of which is herein incorporated by reference in its entirety. The hybrids has among other traits of their normal parent cells, the ability to increase the expression of the angiogenesis inhibitor thrombospondin.

A "switch" to the angiogenic phenotype by fibroblasts cultured from Li-Fraumeni patients coincides with loss of the wild-type allele of the p53 tumor suppressor gene and reduced expression of thrombospondin-1. A novel angiogenesis inhibitor, "angiostatin" is released by the primary tumor mass of a Lewis lung carcinoma. When the primary tumor is present, metastatic tumor growth is suppressed by "angiostatin"; but, after primary tumor removal, the metastases neovascularize and grow. The "angiostatin" activity co-purifies with a 38 kDa plasminogen fragment, as disclosed in O'REILLY et al., "Angiostatin: A Novel Angiogenesis Inhibitor that Mediates the Suppression of Metastases by a Lewis Lung Carcinoma", Cell, 79:315-328 (1994), the disclosure of which is herein incorporated by reference in its entirety. Similarly, endostatin a 20 kDa C-terminal fragment of collagen XVIII prevents the angiogenic switch in pre-malignant lesions, intervening in the rapid expansion of small tumors, or inducing the regression of a large end-stage cancers, as disclosed in O'REILLY et al. (1994), cited above; and BERGERS et al., "Effects of Angiogenesis Inhibitors on Multistage Carcinogenesis in Mice", Science, 284:808-812 (1999), the disclosures of which are herein incorporated by reference in their entireties. Other negative regulators of endothelial proliferation include: platelet factor 4, tissue inhibitors of metalloproteinases, a 16 kDa fragment of prolactin, bFGF/FGF-2 soluble receptor, and TGF $\beta$ , as disclosed in FOLKMAN (1995), cited above, the disclosure of which is herein incorporated by reference in its entirety.---

Please replace the paragraph bridging pages 14-15 with the following new paragraph:

---Glycoproteins need continuous expression of Glc<sub>3</sub>Man<sub>9</sub>GlcNAc<sub>2</sub>-PP-Dol as a pre-requisite for their structural modification. In the dolichol pathway during formation of Glc<sub>3</sub>Man<sub>9</sub>GlcNAc<sub>2</sub>-PP-Dol, the enzyme, Dol-P-Man synthase, is an essential intermediate in the elongation of Man<sub>5</sub>GlcNAc<sub>2</sub>-PP-Dol to Man<sub>9</sub>GlcNAc<sub>2</sub>-PP-Dol, and an allosteric activator of GlcNAc-1-phosphate transferase, as disclosed in CHAPMAN et al., "Structure of the Lipid-linked Oligosaccharides that Accumulate in Class E *thy-1*-negative Mutant Lymphomas", Cell, 17:509-515 (1979); BANERJEE et al., "Amphotomycin: Effect of the Lipopeptide Antibiotic on the Glycosylation and Extraction of Dolichyl Monophosphate in Calf Brain Membranes", Biochemistry, 20:1561-1568 (1981); and KEAN, "Site of Stimulation by Mannosyl-P-dolichol of GlcNAc-lipid Formation by Microsomes of Embryonic Chick Retina", Glycoconjugate J., 13:675-680 (1996), the disclosures of which are herein incorporated by reference in their entireties.---

Please replace the paragraph bridging pages 15-16 with the following new paragraph:

---Regarding CO<sub>2</sub> depletion, N-glycosylation of proteins is increased nearly 3.5-fold, and the K<sub>m</sub> for Dol-P-Man synthase is decreased by ~50% when capillary endothelial cells (an established cell line from the microvasculature of bovine adrenal medulla) were cultured in the absence of CO<sub>2</sub>, as disclosed in BANERJEE et al., "Endothelial Cells from Bovine Adrenal Medulla Develop Capillary-like Growth Patterns in Culture", Proc. Natl. Acad. Sci. USA, 82:4702-4706 (1985); BANERJEE et al., "Microvascular Endothelial Cells from Bovine Adrenal Medulla - A Model for *in vitro* Angiogenesis", Angiogenesis: Models, Modulators and Clinical Applications, pp. 7-18

(1998); and BANERJEE (1998), cited above, the disclosures of which are herein incorporated by reference in their entireties.---

**Please replace the paragraph bridging pages 18-19 with the following new paragraph:**

---In addition, the gene for Dol-P-Man synthase has now been cloned from six different species including *S. cerevisiae*, as disclosed in COLUSSI et al., "Human and *Saccharomyces cerevisiae* Dolichol Phosphate Mannose Synthases Represent Two Class of the Enzyme, but both Function in *Schizosaccharomyces pombe*", Proc Natl Acad Sci (USA), 94: 7873-7878 (1997). It has been shown that Dol-P-Man synthase in *S. cerevisiae* is a structural gene and its mutation is lethal. The Dol-P-Man synthase gene carries a cAMP-dependent protein phosphorylation consensus sequence and its activity is regulated by cAMP-dependent protein kinase-mediated protein phosphorylation signal, as disclosed in ORLEAN et al., "Cloning and Sequencing of the Yeast Gene for Dolichol Phosphate Mannose Synthase, an Essential Proteins", J. Biol. Chem., 263:17499-17507 (1988); MAZHARI-TABRIZI et al., "Cloning and Functional Expression of Glycosyl Transferases from Parasitic Protozoans by Heterologous Complementation in Yeast: the Dolichol Phosphate Mannose Synthase from *Trypanosoma brucei*", Biochem. J., 316:853-858 (1996); ZIMMERMAN et al., "The Isolation of a Dol-P-Man Synthase from *Ustilago maydis* that Functions in *Saccharomyces cerevisiae*", Yeast, 12:765-771 (1996); COLUSSI et al. (1997), cited above; BANERJEE et al. (1987), cited above; and BANERJEE, "Regulation of Mannosylphosphoryldolichol Synthase Activity by cAMP-dependent Protein Phosphorylation", Highlights of Modern Biochemistry, pp. 379-388 (1989), the disclosures of which are herein

incorporated by reference in their entireties. In particular, the sequence data revealed that the Dol-P-Man synthase gene from all species contains one consensus phosphorylation sequence in an area equivalent to Ser-141 in *S. cerevisiae*.---

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CONT

**Please replace the first full paragraph of page 24 with the following new paragraph:**

---Various conditions can interfere with ER function and these are collectively called ER stress. ER stress can arise from a disturbance in protein folding, leading to an accumulation of unfolded or mis-folded proteins in the organelle. Cells respond to the accumulation of unfolded proteins by increasing the transcription of genes encoding ER resident proteins. The information that the capacity of the ER chaperons has been exceeded originates in the ER lumen and is transmitted to the nucleus by an intracellular signaling pathway, the *unfolded protein response (UPR)*. This signaling pathway utilizes several novel mechanisms, including translational attenuation and a regulated mRNA splicing step, as disclosed in PAHL (1999), cited above; REDDY et al., "Assembly, Sorting and Exit of Oligomeric Proteins from the Endoplasmic Reticulum", *BioEssays*, 20:546-554 (1998); and CHAPMAN et al., "Intracellular Signaling from the Endoplasmic Reticulum to the Nucleus", *Annu. Rev. Dev. Biol.*, 14:459-485 (1998), the disclosures of which are herein incorporated by reference in their entirety.---

**Please replace the paragraph bridging pages 23-24 with the following new paragraph:**

---Glycosylation is a means of diversifying a protein without recourse to the genome, and it has the potential to both respond and reflect environmental changes. The endoplasmic reticulum

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(ER) is one of the largest cell organelles, its membrane constituting over one-half of the total membrane in a cell. The ER lumen, the internal space, comprises over 10% of the cell volume. The vast structure has two essential functions. (1) Proteins destined for transport to other organelles, secretion, or expression on the cell surface are synthesized on the ER surface. During translation, they are translocated into the ER lumen through a pore in the ER membrane. Inside the organelle, they are folded, sometimes with the aid of chaperon proteins, and become glycosylated. A quality control mechanism ensures that only correctly folded proteins exit the ER. Incorrectly folded proteins are retained and ultimately degraded. (2) Synthesis of lipids and cholesterol takes place on the cytoplasmic side of the membrane, as disclosed in O'REILLY et al., "Angiostatin: A Novel Angiogenesis Inhibitor that Mediates the Suppression of Metastases by a Lewis Lung Carcinoma", Cell, 79:315-328 (1994); BERGERS et al., "Effects of Angiogenesis Inhibitors on Multistage Carcinogenesis in Mice, Science, 284:808-812 (1999); and PAHL, "Signal Transduction from the Endoplasmic Reticulum to the Cell Nucleus", Physiol. Rev., 79:683-701 (1999), the disclosures of which are herein incorporated by reference in their entireties.---

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**Please replace the paragraph bridging pages 24-26 with the following new paragraphs:**

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---Activation of mammalian *UPR* is characterized in part by increased transcription of at least seven genes encoding ER molecular chaperons. These are Bip/GRP78, as disclosed in LEE, "Mammalian Stress Response: Induction of the Glucose-regulated Protein Family", Curr. Opin. Cell Biol., 4:267-273 (1992), the disclosure of which is herein incorporated by reference in its entirety, as well as induction of C/EBP homologous protein (CHOP), a transcription factor also known as

growth arrest and DNA damage gene product-153 or GADD153, as disclosed in WANG et al., "Signals from the Stressed Endoplasmic Reticulum Induce C/EBP-homologous Protein (CHOP/GADD153)", Mol. Cell. Biol., 16:4273-4280 (1996); and WANG et al., "Cloning of Mammalian Ire1 Reveals Diversity in the ER Stress Responses", EMBO J., 17:5708-5717 (1998), the disclosures of which are herein incorporated by reference in their entireties. Three ER transmembrane signaling proteins that are thought to be the proximal effectors of the *UPR* are Ern1 and 2, PERK, as disclosed in WANG et al., "Cloning of Mammalian Ire1 Reveals Diversity in the ER Stress Responses", EMBO J., 17:5708-5717 (1998); TIRASOPHON et al., "A Stress Response Pathway from the Endoplasmic Reticulum to the Nucleus Requires a Novel Bifunctional Protein Kinase/Endoribonuclease (Ire1p) in Mammalian Cells", Genes Dev., 12:1812-1824 (1998); and HARDING et al., "Protein Translation and Folding are Coupled by an Endoplasmic-reticulum-resident Kinase", Nature, 397:271-274 (1999), the disclosures of which are herein incorporated by reference in their entireties.

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cont.*

In principle, the mechanism underlying *UPR*-induced ER-stress condition could indirectly impede cell-cycle progression by interfering with the proper maturation of growth factor receptors or other modulators of mitogenic signaling, as disclosed in CAI et al., "Protein Translation and Folding are Coupled by an Endoplasmic-reticulum-resident Kinase", Nature, 397:271-274 (1998), the disclosure of which is herein incorporated by reference in its entirety. Alternatively, ER stress may directly induce checkpoint response that prevents cells from completing their cell division cycle under conditions that compromise the proper folding and assembly of proteins response, as disclosed in BREWER et al., "Mammalian Unfolded Protein Response Inhibits Cyclin D1 Translation and

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Cell-cycle Progression", Proc. Natl. Acad. Sci. (USA), 96:8505-8610 (1999); and NAKAGAWA et al., "Caspase-12 Mediates Endoplasmic-reticulum-Specific Apoptosis and Cytotoxicity by Amyloid- $\beta$ ", Nature, 403:98-103 (2000), the disclosures of which are herein incorporated by reference in their entireties. Since the late 1970s there has been a clear link between sugar metabolism and the *UPR*, as disclosed in POUYSSEGUR et al., "Induction of Two Transformation-sensitive Membrane Polypeptides in Normal Fibroblasts by a Block in Glycoprotein Synthesis or Glucose Deprivation", Cell, 11:941-947 (1977); SHIU et al., "Glucose Depletion Accounts for the Induction of Two Transformation-sensitive Membrane Proteins in Rous Sarcoma Virus-transformed Chick Embryo Fibroblasts", Proc. Natl. Acad. Sci. (USA) 74:3840-3844 (1977); and PELUSO et al., "Infection with Paramyxoviruses Stimulates Synthesis of Cellular Polypeptides that are also Stimulated in Cells Transformed by Rous Sarcoma Virus or Deprived of Glucose", Proc. Natl. Acad. Sci. (USA), 75:6120-6124 (1978); GETHING et al., "Protein Folding in the Cell", Nature, 355:33-45 (1992); PAHL et al., "A Novel Signal Transduction Pathway from the Endoplasmic Reticulum to the Nucleus is Mediated by Transcription Factor NF-kappa B", EMBO J., 14:2580-2588 (1995); and WATOWICH et al., "Complex Regulation of Heat Shock- and Glucose-responsive Genes in Human Cells", Mol Cell Biol., 8:393-405 (1988), the disclosures of which are herein incorporated by reference in their entireties.---

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**Please replace the paragraph bridging pages 41-42 with the following new paragraph:**

---Morphological evaluation has indicated that cell proliferation and differentiation are mutually exclusive events. Survival of cells in low-serum concentrations (i.e., 1% (v/v)) and without

*Q16*

processing through the proliferative pathway, hypothesized that the low-proliferative response may either be mediated by a depletion in serum growth-factor(s) concentrations or due to an appearance of anti-metabolites during their growth. This may also be true for cultures exhibiting putative capillary-lumen formation when maintained in 2% (v/v) serum for a relatively long period of time.

Earlier transmission electron microscopic studies established that these cells synthesize and secrete basal lamina structures and engage in transcytosis, a characteristic ultrastructural and functional

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*cont* combination of exocytosis and endocytosis across the thin endothelial cell processes, as discussed in BANERJEE et al., "Endothelial Cells from Bovine Adrenal Medulla Develop Capillary-like Growth Patterns in Culture", Proc. Natl. Acad. Sci. (USA), 82:4702-4706 (1985), the disclosure of which is herein incorporated by reference in its entirety. Scanning electron microscopy has now documented a distinct surface morphology during their proliferation as well as when they were undergoing differentiation into capillary-like structures.---

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**Please replace the paragraph bridging pages 42-43 with the following new paragraph:**

---Nearly, 70% of the synchronous cell population entered into apoptosis after exposure to tunicamycin for 32 hours as indicated in Figs. 13B-13D, discussed in more detail below. This was certainly not due to cell toxicity, because a similar growth reduction has been observed at a wide range of tunicamycin concentrations (i.e., 0.5  $\mu$ g/ml - 5.0  $\mu$ g/ml), and the effect was reversible, as discussed in MARTÍNEZ et al., "N-glycosylation Inhibition on Endothelial Cell Proliferation and Viability", FASEB J., 12:231a (1998), the disclosure of which is herein incorporated by reference in its entirety. In addition, more than 70% of total adherent cells were viable under such conditions.

The presence of a large population of apoptotic cells may suggest a relationship between the onset of tunicamycin-induced apoptosis and the cell cycle, where the N-glycans play a critical role in cell cycle check points. These results indicate that induction of apoptosis is the primary cause for the inhibition of capillary endothelial cell proliferation by tunicamycin. High level of immunopositive Factor VIII:C in the conditioned media from cells treated with tunicamycin confirmed that intracellular accumulation of unglycosylated Factor VIII:C might serve as an apoptotic signal causing changes in cell permeability and consequently osmotic lysis. Deglycosylation of Factor VIII:C in fact, by N-glycanase digestion has been shown to eliminate the biological activity of Factor VIII:C, (data not shown).---

**Please replace the paragraph bridging pages 51-52 with the following new paragraph:**

---These cells have been characterized in BANERJEE, "Angiogenesis: Characterization of a Cellular Model", Puerto Rico Health Science Journal, 17:327-333 (publication date unknown); BANERJEE et al., "Endothelial Cells from Bovine Adrenal Medulla Develop Capillary-like Growth Patterns in Culture", Proc. Natl. Acad. Sci (USA), 82:4702-4706 (1985); BANERJEE et al., "Microvascular Endothelial Cells from Adrenal Medulla - a Model for *in vitro* Angiogenesis", Models, Modulators, and Clinical Applications, pp. 7-18 (1998); BANERJEE et al., "Is Asparagine-linked Protein Glycosylation an Obligatory Requirement for Angiogenesis?", Indian J. Biochem. Biophys., 30:389-394 (1993); YOUDIM et al., "Isolated Chromaffin Cells from Adrenal Medulla Contain Primarily Monoamine Oxidase B", Science, 224:619-621 (1984); YOUDIM et al., "Steroid Regulation of Monoamine Oxidase Activity in the Adrenal Medulla", FASEB J., 3:1753-1759

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(1989); BANERJEE et al., "Expression of Blood Clotting Factor VIII:C Gene in Capillary Endothelial Cells", FEBS Letts., 306:33-37 (1992); MARTÍNEZ et al., "Expression of Glc<sub>3</sub>Man<sub>9</sub>GNAc<sub>2</sub>-PP-Dol is a Pre-requisite for Capillary Endothelial Cell Proliferation", Cell Molec. Biol., 45:137-152 (1999); BANERJEE et al., "Biphasic Estrogen Response on Bovine Adrenal Medulla Capillary Endothelial Cell Adhesion, Proliferation and Tube Formation", Mol. Cell Biol., 177:97-105 (1997); BANERJEE, "Microenvironment of Endothelial Cell Growth and Regulation of Protein N-glycosylation", Indian J. Biochem. Biophys., 25:8-13 (1998), the disclosures of which are herein incorporated by reference in their entireties.---

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Cont.  
Please replace the second full paragraph on page 53 with the following new paragraph:

---The above described capillary endothelial cells were maintained in EMEM containing 10% (v/v) heat-inactivated FBS (30 minutes @ 56°C), glutamine (2 mM), penicillin (50 units/ml), streptomycin (50 µg/ml), and nystatin (100 units/ml), i.e., a complete EMEM at 37°C in a humidified CO<sub>2</sub> incubator (5% (v/v) CO<sub>2</sub> /95% (v/v) air) in tissue culture flasks or dishes without collagen underlay or other extracellular matrix components, as described before in BANERJEE et al., "Endothelial Cells from Bovine Adrenal Medulla Develop Capillary-like Growth Patterns in Culture", Proc. Natl. Acad. Sci. (USA), 82:4702-4706 (1985), the disclosure of which is herein incorporated by reference in its entirety. Cells were subcultured once a week unless otherwise mentioned.---

**Please replace the first full paragraph of page 54 with the following new paragraph:**

---For scanning electron microscopy,  $5 \times 10^3$  cells were cultured on 13 mm round Thermanox coverslips in 12 well clusters (COSTAR) in complete EMEM. Cells were synchronized as mentioned above and cultured in EMEM containing either 2% (v/v) FBS (control), 10% (v/v) FBS or 2% (v/v) FBS plus 1  $\mu$ g/ml tunicamycin. The coverslips were removed at specified times and the cells were fixed in 2.5% (v/v) glutaraldehyde for 30 minutes. The cells were washed three times (10 minutes each) with Millonig (1961) isotonic phosphate buffer, pH 7.3, and treated with 1% (w/v) osmium tetroxide for 15 minutes. See MILLONIG, "Advantages of a Phosphate Buffer for Osmium Tetroxide Solutions in Fixation", *J. Appl. Physics*, 32:1637 (1961), the disclosure of which is herein incorporated by reference in its entirety. After washing with isotonic phosphate buffer, pH 7.3 with three changes, the specimens were dehydrated with 30%, 60% and 90% (10 minutes each), and finally with 100% (v/v) acetone in three changes. In the final step, the specimens were treated with 1:1 mixture of 100% (v/v) acetone and Peldry II for 1 hour and then changed to 100% (v/v) Peldry II for 30 minutes. The specimens were cooled below 23°C (18° to 20°C), placed under low vacuum (over night), mounted on a sample mount, coated with metal in E5200 Auto Sputter (gold film thickness 200 nm) and examined in Autoscan ETEC Scanning Electron Microscope.---

**Please replace the paragraph bridging pages 55-56 with the following new paragraph:**

---Regarding flow cytometry, assessment of the cell cycle was determined by propidium iodide staining of fixed cell nuclei followed by flow cytometry in a FacSort (Beckton Dickinson) according to the method of Krishan (1975) as modified by Vindelov (1977), as discussed in

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KRISHAN, "Rapid Flow Cytofluorometric Analysis of Mammalian Cell Cycle by Propidium Iodide Staining", J. Cell Biol., 66:188-193 (1975); and VINDELOV, "Flow Cytometric Analysis of Nuclear DNA in Cells from Solid Tumors and Cell Suspensions", Virchows Arch (B), 24:227-231 (1977), the disclosures of which are herein incorporated by reference in their entireties. Briefly, cells were trypsinized, and pelleted by centrifugation at 900 rpm in a bench top centrifuge (Sorvall T6000B). After washing twice with PBS the cell pellet was resuspended in propidium iodide-buffer (PI-buffer; 10 mM Tris-HCl, pH 8.0 containing 10 mM NaCl, 0.7 µM PI, 700 units/liter RNase, and 0.1% (v/v) NP-40), kept in ice for 30 minutes and then fixed with 2% (v/v) paraformaldehyde. After FacSort calibration, 3000 events were counted per sample. Acquisition and analysis of data were carried out by using Cell Quest flow cytometry software (Macintosh-based). Gates: M1 = G0/G1, M2 = S, M3 = G2 + M, M4 = apoptosis.---

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Please replace the first full paragraph on page 60 with the following new paragraph:

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---*Culturing of capillary endothelial cells*: The stock culture of capillary endothelial cells was maintained in EMEM containing 10% (v/v) heat-inactivated FBS (30 minutes @ 56°C), glutamine (2 mM), penicillin (50 units/ml), streptomycin (50 µg/ml), and nystatin (100 units/ml), i.e., a complete EMEM at 37°C in a humidified CO<sub>2</sub> incubator (5% (v/v) CO<sub>2</sub>/95% (v/v) air) in tissue culture flasks or dishes without collagen underlay or other extracellular matrix components, as described before in BANERJEE et al., "Endothelial Cells from Bovine Adrenal Medulla Develop Capillary-like Growth Patterns in Culture", Proc. Natl. Acad. Sci. (USA), 82:4702-4706 (1985), the

disclosure of which is herein incorporated by reference in its entirety. Cells were subcultured once a week unless otherwise mentioned.---

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cont*

**Please replace the paragraph bridging pages 60-61 with the following new paragraph:**

---*Scanning Electron Microscopy of Capillary Endothelial Cells:*  $5 \times 10^3$  cells were cultured on 13 mm round Thermanox coverslips in 12 well clusters (COSTAR) in complete EMEM. Cells were synchronized as mentioned above and cultured in EMEM containing either 2% (v/v) FBS (control), 10% (v/v) FBS or 2% (v/v) FBS plus 1  $\mu$ g/ml tunicamycin. The coverslips were removed at specified times and the cells were fixed in 2.5% (v/v) glutaraldehyde for 30 minutes. The cells were washed three times (10 minutes each) with Millonig (1961) isotonic phosphate buffer, pH 7.3, and treated with 1% (w/v) osmium tetroxide for 15 minutes. See MILLONIG, "Advantages of a Phosphate Buffer for Osmium Tetroxide Solutions in Fixation", *J. Appl. Physics*, 32:1637 (1961), the disclosure of which is herein incorporated by reference in its entirety. After washing with isotonic phosphate buffer, pH 7.3 with three changes, the specimens were dehydrated with 30%, 60% and 90% (10 minutes each), and finally with 100% (v/v) acetone in three changes. In the final step, the specimens were treated with 1:1 mixture of 100% (v/v) acetone and Peldry II for 1 hour and then changed to 100% (v/v) Peldry II for 30 minutes. The specimens were cooled below 23°C (18° to 20°C), placed under low vacuum (over night), mounted on a sample mount, coated with metal in E5200 Auto Sputter (gold film thickness 200 nm) and examined in Autoscan ETEC Scanning Electron Microscope.---